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NOTES ON THE ACTIVITY OF EARTHWORMS

III. The conditioning effect of earthworms on the surrounding soil

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Introduction

In an earlier study on the influence of different factors on diapause in the earthworms *Allolobophora caliginosa* (2), the hypothesis was put forward that diapause in earthworms is induced by a product primarily excreted by the worms themselves, and most likely changed by micro-organisms.

Acting on this assumption, it was considered necessary to collect more evidence which might either confirm or contradict this hypothesis.

If a relatively great number of earthworms is kept together in soil (up to 200 in a 10 l pail), they do quite well for some weeks, dependent on temperature; afterwards they suddenly grow sluggish and the greater part of *Allolobophora caliginosa* goes into diapause, whereas most *Lumbricus rubellus* die.

If the worms are removed at the first signs of this change in behaviour, the remaining soil proves to be unhealthy for other, even freshly caught worms. This quality is conserved for months at 2°C, but disappears in 6 weeks at room temperature (20°–25°C), if the soil is kept moist. Heating the moist soil for three hours at 120°C increases the unfavourable effect on earthworms. Washing the soil by mixing it with an equal weight of distilled water and removing the water by a Buchner-funnel, has no effect.

In this paper soil, in which worms have been kept for as long as they could reasonably stand, is called 'conditioned' soil, while a difference is made between '*caliginosa* soil' and '*rubellus* soil', if it has been conditioned by *A. caliginosa* or *L. rubellus* resp.

Observations

A quantity of a good sandy garden-soil was thoroughly mixed and sieved. Parts of it were conditioned by *A. caliginosa* and *L. rubellus* resp., the remainder was kept under the same conditions as those under which conditioning took place.

As earthworms in a pail with soil are not regularly distributed in the soil, but show a pronounced preference for certain spots, only that part of the soil in which the worms aggregate will be fully conditioned. After mixing the total contents of the pail, the conditioned soil must have already been diluted, moreover it had been stored at 2°C for three weeks prior to the experiments. Nevertheless, in this paper, this soil is called 100% conditioned.

Mixtures of conditioned and original (fresh) soil were made, with increasing percentages of conditioned soil. Five breeding cells ('flats'), as described elsewhere (1), were filled with each of these mixtures. Per flat 2 specimens of *A. caliginosa* and *L. rubellus* resp. were added. The behaviour of the worms was regularly observed. The experiment lasted for 62 days. The average time of survival of the group of 10 worms per mixture was calculated. The day before that on which death of a certain individual

was noted, was taken as its last day of life. The time of survival of the individuals still alive at the end of the experiment is > 62.

In the tables 1 and 2 the data have been collected on *caliginosa*-soil and *rubellus*-soil resp. However, figures obtained in experiments with earthworms always have a strong tendency to be irregular, unless a great number of individuals is involved, which demands much room, material and labour.

It is evident from table 1, that with increasing percentages of *caliginosa*-soil, the average time of survival of *rubellus* is very short. Table 2 shows that conditioning by *rubellus* itself has some influence on this worm, but the data are too irregular to allow further conclusions to be drawn.

More interesting is the behaviour of *A. caliginosa* in soils with increasing conditioning by the same species (table 1, fig. 1). The onset of diapause is earlier, but from 50%-caliginosa-soil onwards mortality is high, some worms dying without first going into diapause, others after they have already reached the resting state.

This high death-rate of freshly caught worms which were put into more or less conditioned soil was most remarkable. We did not notice any repellant effect of the conditioned soils, the worms burrowing into it just as they did into fresh soil. The rate of conditioning of the fresh and the 25%-conditioned soil (upper two diagrams of fig. 1) at the moment of general diapause, must have been at least as high, but possibly higher, as the conditioned soil with which the experiment was started. Yet, 30 days after almost general diapause had been reached in fresh- and in 25%-*caliginosa*-soil,

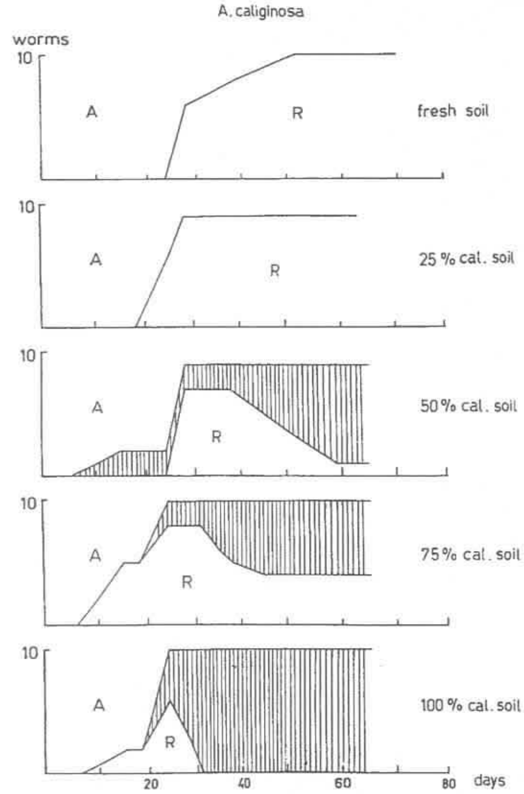

FIG. 1. The effect of different degrees of soil-conditioning produced by *A. caliginosa*, on diapause and death-rate of the same species.

no death had occurred, whereas 30 days after starting the experiment in 100%-*caliginosa*-soil all animals were dead.

In *rubellus*-soil, mortality of *A. caliginosa* was low and irregularly distributed over the treatments (table 2). As for diapause, this was strongly influenced by the conditioning of the soil by *L. rubellus* (fig. 2), but the effect of conditioning by *L. rubellus* seems to be different from that by *A. caliginosa*.

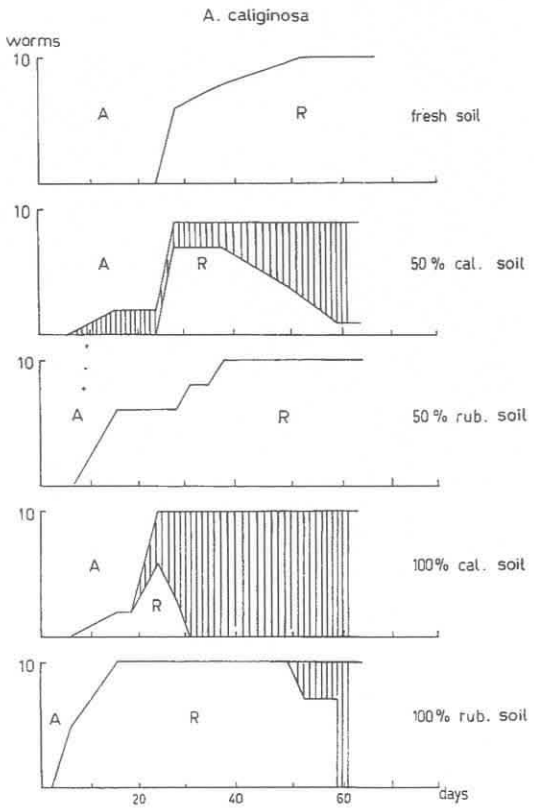

FIG. 2. The effect of different degrees of soil-conditioning produced by *A. caliginosa* and *L. rubellus* on diapause and death rate of *A. caliginosa*.

TABLE 1. Average time of survival in days of 10 specimens each of *A. caliginosa* and *L. rubellus* in a soil conditioned by *A. caliginosa*.

Caliginosa soil %	fresh soil %	*A. caliginosa* average time of survival	*A. caliginosa* number surviving	*L. rubellus* average time of survival	*L. rubellus* number surviving
0	100	> 62.0	10	42.8	–
25	75	> 62.0	10	26.4	–
50	50	> 46.3	2	28.6	–
75	25	> 44.4	4	14.1	–
100	0	27.3	–	7.8	–

TABLE 2. Average time of survival of 10 specimens each of *L. rubellus* and *A. caliginosa* in a soil conditioned by *L. rubellus*.

Rubellus soil %	fresh soil %	*L. rubellus* average time of survival	*L. rubellus* number surviving	*A. caliginosa* average time of survival	*A. caliginosa* number surviving
0	100	42.8	–	> 62.0	10
25	75	28.1	–	> 55.2	8
50	50	22.0	–	> 62.0	10
75	25	30.5	–	> 49.6	8
100	0	32.9	–	> 59.0	7

DISCUSSION

From the data given, it is likely that diapause primarily is induced by a substance excreted by the earthworms themselves. Whether this substance has to be changed by micro-organisms before it is harmful, remains to be studied. It is obvious that if the inducement of going into diapause is too strong or too sudden, *A. caliginosa* cannot react properly to it, but dies, either directly or shortly after the onset of diapause. In *L. rubellus* the same stimulant causes death quickly. It is not certain whether the active substances derived from *L. rubellus* and *A. caliginosa* are the same, or if the differences observed, depend on differences in concentration only.

Isolating possible diapause-inducing substances is complicated by the fact that a too strong stimulant causes death instead of diapause. Since death is not a very specific reaction, isolates have to be diluted before they can be tried on earthworms. So far, diapause could not be induced, or recognised as such, in vitro. Therefore diluted isolates have to be added to soil, which again, is complicating the procedure, as microbial activity cannot be excluded. Before any conclusions may be drawn and purification of the isolates can proceed, a normal diapause has to be observed, preferably both in the experiment and in the controls.

In the field, the alimentary tract of earthworms in diapause is always empty. During starvation in the laboratory it takes a worm 8–10 days to empty its gut. This shows, that in the field the inducement for going into diapause must be strong enough to make the animal stop feeding at least 10 days before the onset of diapause. Diapause in earthworms cannot simply be a prolonged resting state, as regeneration or the growth of new segments only takes place during diapause. Red-pigmented earthworm species which cannot go into diapause, do not regenerate nor grow new segments at the posterior end (3).

Considering all this, it is not surprising that a worm needs rather a long time to adapt itself to a stimulant before being able to go into diapause.

So far the only chemically pure substance with which a seemingly normal diapause could be induced, is lysine.

200 g of a garden soil with 8 g of organic matter in all was percolated daily for 5 days with 100 ml of a sterilised $1^0/_{00}$-solution of l-lysine dihydrochloride, brought to pH 6.8 with sodium hydroxide. Between percolations, the wet soil column was kept at 20–25°C in the percolation tubes. In all, 0.5 g lysine-dihydrochloride (= 0.335 g lysine) was used; the amount directly lost by the percolate was not determined.

Another 200 g of the same soil was percolated in the same way with distilled water only. The pH of the last percolates was 6.2 and 6.1 for lysine and water resp.

The soils were dried at room temperature for four days. After thorough mixing 5 flats were filled with each of the two soils. To each flat 2 specimens of *A. caliginosa* were added.

After 24, 27, 29 and 36 days, in the 'lysine-soil' 4, 6, 8 and 9 worms were in diapause resp., whereas in the 'distilled-water-soil.' only one was.

Evidently this experiment does not say much of the true character of the diapause-inducing agent. Experiments in this field are continued.

References

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